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CCR2 Expression Determines T1 Versus T2 Polarization During Pulmonary Cryptococcus neoformans Infection

Tim R. Traynor,* William A. Kuziel, † Galen B. Toews,* and Gary B. Huffnagle 2*

Pulmonary clearance of the encapsulated yeast Cryptococcus neoformans requires the development of T1-type immunity. The objective of this study was to determine the role of CCR2 in leukocyte recruitment and development of T1-type cell-mediated immunity during pulmonary C. neoformans infection. Intratracheal inoculation of C. neoformans into CCR2 knockout (CCR2−/−) mice produced a prolonged pulmonary infection (5000-fold CFU at 6 wk compared with CCR2+/+ mice) and significant dissemination to the spleen and brain (160- and 800-fold greater). In addition, CCR2 deficiency resulted in significantly reduced recruitment of macrophages (weeks 1–3) and CD8+ T cells (weeks 1–2) into the lungs. The immune response in CCR2−/− mice was characterized by chronic pulmonary eosinophilia, crystal deposition in the lungs, pulmonary leukocyte production of IL-4 and IL-5 but not IFN-γ, lack of anticytotoxic delayed-type hypersensitivity, and high levels of serum IgE. These results demonstrate that expression of CCR2 is required for the development of a T1-type response to C. neoformans infection and lack of CCR2 results in a switch to a T2-type response. Thus, CCR2 plays a critical role in promoting the development of T1- over T2-type immune responses in the lung following cryptococcus infection. The Journal of Immunology, 2000, 164: 2021–2027.

The encapsulated yeast Cryptococcus neoformans is acquired via the respiratory tract, and T1-type cell-mediated immunity (CMI) 1 is critical for pulmonary clearance of this opportunistic pathogen (1). T1-type CMI to pulmonary C. neoformans infection is characterized by (1) the production of IFN-γ (2); a substantial influx of macrophages, lymphocytes, and neutrophils into the lungs; and (3) the development of Ag-specific delayed-type hypersensitivity (DTH) to C. neoformans (1). The T1-type response requires CD4+ and CD8+ T cells in addition to the production of the cytokines TNF-α, IL-12, and IFN-γ (1). Passive immunization of C. neoformans-infected mice with anti-capsular Abs also requires CD4+ T cells and IFN-γ for clearance of the infection (2).

Leukocyte recruitment into the lungs during a protective T1 response to pulmonary C. neoformans infection is not only T cell dependent, but also requires monocyte chemoattractant protein-1 (MCP-1) (3). MCP-1 is a C-C chemokine that can be produced by most cell types when stimulated by microbial products or certain cytokines and is chemotactic for monocytes and T lymphocytes (4, 5). Intratracheal inoculation with C. neoformans has been shown to stimulate production of MCP-1 in the lungs (3). Neutralization of MCP-1 after immunity develops results in a 95% reduction in macrophage recruitment and abrogation of cryptococcal clearance (3). Thus, MCP-1 plays a critical role in the effector phase of T1-type CMI to pulmonary C. neoformans infection.

The primary receptor for MCP-1 is CCR2 (6). CCR2 is expressed by monocytes, activated T cells, B cells, and NK cells (7). CCR2 knockout mice have severe deficits in macrophage recruitment in response to either antigenic or nonantigenic challenge (8–10). In addition, there is evidence to suggest that the T1-type cytokine response may be impaired (8). Our objective was to determine the role of CCR2 in leukocyte recruitment and development of T1-type CMI during pulmonary C. neoformans infection.

Materials and Methods

Mice

CCR2−/− mice (B6129F2/J; The Jackson Laboratory, Bar Harbor, ME) and CCR2+/− mice (129B6F2-Cmkbr2tm1Kuz (10); University of Michigan breeding colony) were housed under specific pathogen-free conditions in enclosed filter top cages. Clean food and water were given ad libitum. The mice were handled and maintained using microisolator techniques with daily veterinarian monitoring. Bedding from the mice was transferred weekly to cages of uninfected sentinel mice that were subsequently bled at weekly intervals and found to be negative for Abs to mouse hepatitis virus, Sendai virus, and Mycoplasma pulmonis. Mice were 8–16 wk of age at the time of infection and there were no age-related differences in the responses of these mice to C. neoformans infection.

C. neoformans

C. neoformans strain 52D was obtained from the American Type Culture Collection (no. 24067-E; Manassas, VA). For infection, yeast were grown to stationary phase (48–72 h) at 37°C in Sabouraud dextrose broth (1% neopeptone and 2% dextrose; Difco, Detroit, MI) on a shaker. The cultures were then washed in nonpyrogenic saline, counted on a hemocytometer, and diluted to 3.3 × 105 CFU/ml in sterile nonpyrogenic saline.

Surgical intratracheal inoculation

Mice were anesthetized by i.p. injection of pentobarbital (0.074 mg/g weight of mouse) and restrained on a small surgical board. A small incision was made through the skin over the trachea and the underlying tissue was separated. A 30-gauge needle was bent and attached to a tuberculin syringe
filled with diluted \textit{C. neoformans} culture. The needle was inserted into the trachea, and 30 \(\mu\)l of inoculum (10^6 CFU) was dispensed into the lungs. The needle was removed and the skin closed with cyanoacrylate adhesive. The mice recovered with minimal visible trauma.

\textbf{CFU assay}

For lung CFU, small aliquots were collected from lung digests (described below). For brain and spleen CFU, the organs were excised, placed in 2 ml sterile water, and homogenized. Ten-microliter aliquots of the lungs, brain, and spleen were plated out on Sabouraud dextrose agar plates in duplicate 10-fold dilutions and incubated at room temperature. \textit{C. neoformans} colonies were counted 2 to 3 days later, and the number of CFU was calculated on a per organ basis.

\textbf{Lung leukocyte isolation}

Individual lungs were excised, minced, and enzymatically digested for 30 min in 15 ml of digestion buffer (RPMI, 5\% FCS, antibiotics, 1 mg/ml collagenase, and 30 \(\mu\)g/ml DNase). The cell suspension and undigested fragments were further dispersed by drawing up and down through the bore of a 10-ml syringe. The total cell suspension was then pelleted, and the erythrocytes were lysed by resuspending them in ice-cold NH_4Cl buffer (0.83\% NH_4Cl, 0.1\% KHCO_3, and 0.037\% Na_EDTA, pH 7.4). Tenfold excess of media was added to return the solution to isotonicity. The isolated leukocytes were repelleted and resuspended in complete media. Total lung leukocyte numbers were assessed in the presence of trypsin blue using a hemocytometer; viability was \(>85\%). Subsets of isolated leukocytes (neutrophils, eosinophils, macrophages, and total lymphocytes) were determined by Wright-Giemsa staining of samples cytospin onto slides.

\textbf{Flow-cytometric analysis of lymphocyte subsets}

Leukocytes (5 \(\times\) 10^7) were incubated for 30 min on ice with staining buffer (FA buffer, 0.1\% NaN_3, 1\% FCS; Difco). Each sample was incubated with 1) 0.12 \(\mu\)g of Cy-Chrome-labeled anti-CD45 (30-F11; PharmIngem, San Diego, CA) and either 2) 0.25 \(\mu\)g each of FITC-labeled anti-CD4 (RM4-5) and PE-labeled anti-CD8 (53-6.7) or 3) 0.25 \(\mu\)g of FITC-labeled anti-B220 (RA3-6B2). The samples were washed in staining buffer and fixed in 2.5\% paraformaldehyde in buffered saline. Stained samples were stored in the dark at 4\(^\circ\)C until analyzed by flow cytometry (Coultier Elite ESP, Palo Alto, CA). Samples were gated for CD45-positive cells and then analyzed for staining by the specific FITC- and PE-labeled antilymphocyte markers.

\textbf{Histology}

Following euthanasia and before removal, the lungs were fixed by inflation with 1 ml of 10\% neutral buffered Formalin. The fixed lung specimens were stored in 10\% neutral buffered Formalin until dehydrated in 70\% ethanol and paraffin embedded. Sections (5 \(\mu\)m) were cut, deparaffinized, stained with hematoxylin and eosin, and viewed by light microscopy.

\textbf{Lung leukocyte culture and cytokine production}

Isolated leukocytes (15 \(\times\) 10^7) were cultured in six-well plates with 3 ml of complete medium at 37\(^\circ\)C and 5\% CO_2 without any additional stimulus. Supernatants were harvested at 24 h and assayed for IL-4, IL-5, and IFN-\(\gamma\) production by sandwich ELISA using the manufacturer’s instructions supplied with the cytokine-specific kits (OptEIA; Pharmingen).

\textbf{DTH response assay}

Mice were tested for the development of DTH-mediating \(T\) cell immunity using a modification of a previously described footpad DTH assay (11). The Ag preparation for the assay, \textit{C. neoformans} filtrate Ag, was prepared by concentrating and dialyzing the supernatant from a \textit{C. neoformans} asparaginase broth culture. The major protein in this preparation is a mannoprotein that cross-reacts with all strains and serotypes of \textit{C. neoformans}. For each mouse tested, the hind right footpad was injected with 20 \(\mu\)l of \textit{C. neoformans} filtrate Ag and the hind left footpad injected with 20 \(\mu\)l of dialyzed asparagine broth/2\% BSA. After 48 h, the thickness of each footpad was measured using a micrometer. The swelling in the right footpad will be determined by subtracting the measurement of the right footpad from the measurement of the left. Uninfected mice were also challenged as a negative control for the assay.

\textbf{Bronchoalveolar lavage (BAL)}

Mice were lavaged after cannulation of the trachea with polyethylene tubing (PE50), which was attached to a 25-gauge needle on a tuberculin syringe. The lungs were lavaged twice with 0.8 ml of PBS containing 5 mM EDTA. The recovered fluid (1.3–1.4 ml total) was spun at 1500 rpm, and the supernatant was removed and stored at \(-20\)\(^\circ\)C until analyzed for MCP-1 by ELISA (OptEIA; Pharmingen).

\textbf{Statistics}

Statistical significance was calculated using a \(t\) test with significance being \(p < 0.05\) for a single comparison. All values are reported as mean \(\pm\) SE.

\textbf{Results}

\textbf{CCR2 deficiency results in an uncontrolled pulmonary \textit{C. neoformans} infection}

The role of CCR2 in protective immunity against pulmonary \textit{C. neoformans} infection was initially determined by assessing pulmonary clearance and extrapulmonary dissemination after intra-tracheal inoculation of \textit{C. neoformans} strain 52D. By week 1 postinfection, the pulmonary cryptococcal burden increased 350-fold and remained elevated through week 2 in both CCR2+/+ and CCR2−/− mice (Fig. 1). CCR2-expressing mice began to clear the infection at about week 3 and, by week 6, lung CFU had decreased by over 300-fold. In contrast, CCR2-deficient mice failed to control the infection between weeks 3–6, and lung CFU were 5000-fold greater in CCR2−/− mice than in CCR2+/+ mice at week 6. These data show that CCR2 expression is required for clearance of a pulmonary \textit{C. neoformans} infection between weeks 3 and 6 postinfection.

The inability to express CCR2 also resulted in enhanced dissemination of the \textit{C. neoformans} to peripheral organs (Fig. 1). Over the first 3 wk of infection, CCR2+/+ and CCR2−/− mice had similar numbers of \textit{C. neoformans} in the spleen and brain. However, by week 6, disseminated growth of \textit{C. neoformans} was significantly greater (160- and 800-fold higher CFU per organ, respectively) than in CCR2+/+ mice. Thus, CCR2 is also required to control disseminated growth of \textit{C. neoformans} in extrapulmonary organs.
MCP-1 is produced in the lungs of CCR2-deficient mice

Because MCP-1 is important in the clearance of a pulmonary C. neoformans infection, our next objective was to determine whether the absence of CCR2 diminished the production of MCP-1 in the lung. MCP-1 production by pulmonary leukocytes was assayed in vitro following isolation by mincing and enzymatic digestion. Leukocytes were isolated from infected lungs and cultured for 24 h without any additional stimulus. Culture supernatants were then harvested and analyzed for MCP-1 production by ELISA. A small, early influx of leukocytes (week 1) was observed by assessing the phenotypes of the leukocytes involved in the response. A small, early influx of leukocytes (week 1) was observed in CCR2+/+ mice, but was absent in CCR2−/− mice (Fig. 3). However, there was a dramatic increase in lung leukocyte numbers in both CCR2+/+ and CCR2−/− mice at 2 wk postinfection. By week 6, total lung leukocyte numbers had declined in CCR2+/+ mice, correlating with resolution of the infection. In contrast, lung leukocyte numbers remained elevated in CCR2−/− mice. Thus, the absence of CCR2 does not result in an overall defect in lung leukocyte recruitment in response to pulmonary C. neoformans infection.

Lack of CCR2 results in defective macrophage recruitment and the development of chronic eosinophilia

Although both CCR2+/+ and CCR2−/− mice responded to infection by developing a vigorous inflammatory response, cytological analysis revealed differences in the leukocyte subsets recruited.

FIGURE 2. MCP-1 in leukocyte culture supernatants and in BAL fluid (BALF) from C. neoformans-infected CCR2+/+ and CCR2−/− mice. A, Total lung leukocytes were isolated from individual lungs following mincing and enzymatic digestion of the lungs (described in Materials and Methods) and cultured for 24 h without any additional stimulus. Culture supernatants were then harvested and analyzed MCP-1 production by ELISA. *, p < 0.05 for CCR2+/+ vs CCR2−/−; n = 8 for each group (two separate experiments of four mice each); values are means ± SEM. B, BALF was analyzed for MCP-1 by ELISA 1 wk postinfection. *, p < 0.05 for week 1 infected vs uninfected CCR2+/+ or CCR2−/−; **, p < 0.05 for week 1 infected CCR2−/− vs week 1 CCR2+/+; n = 5 for each group of infected mice and n = 9 for each group of week 1 mice; values are means ± SEM.

FIGURE 3. Total leukocyte recruitment into the lungs of CCR2+/+ and CCR2−/− mice following pulmonary C. neoformans infection. Mice were infected as described in Fig. 1. Total lung leukocytes were isolated from individual lungs, as described in Materials and Methods. Week 0 data are from a parallel cohort of uninfected mice. *, p < 0.05 for CCR2+/+ vs CCR2−/−; n = 8 for each group (two separate experiments of four mice each); values are means ± SEM.

Lack of CCR2 does not inhibit the development of a pulmonary inflammatory response to C. neoformans infection

The next objective was to determine whether pulmonary leukocyte recruitment was diminished in the absence of CCR2, thereby contributing to the decreased clearance of C. neoformans. Leukocyte recruitment was determined by quantifying leukocyte numbers and by assessing the phenotypes of the leukocytes involved in the response. A small, early influx of leukocytes (week 1) was observed in CCR2+/+ mice, but was absent in CCR2−/− mice (Fig. 3). However, there was a dramatic increase in lung leukocyte numbers in both CCR2+/+ and CCR2−/− mice at 2 wk postinfection. By week 6, total lung leukocyte numbers had declined in CCR2+/+ mice, correlating with resolution of the infection. In contrast, lung leukocyte numbers remained elevated in CCR2−/− mice. Thus, the absence of CCR2 does not result in an overall defect in lung leukocyte recruitment in response to pulmonary C. neoformans infection.

FIGURE 4. Leukocyte subset recruitment after pulmonary C. neoformans infection in CCR2+/+ and CCR2−/− mice. Total lung leukocyte suspensions were prepared as outlined in Materials and Methods. Samples of leukocyte suspensions from infected mice were cytospun onto slides and stained with Wright-Giemsa stain for visual quantification of neutrophils, eosinophils, macrophages, and lymphocytes (described in Materials and Methods). *, p < 0.05 for CCR2+/+ vs CCR2−/−; n = 8 for each group (two separate experiments of four mice each); values are means ± SEM.
Lack of CCR2 results in delayed CD8\(^+\) T cell recruitment. Lack of CCR2 expression results in defective macrophage recruitment and development of chronic eosinophilia in response to pulmonary *C. neoformans* infection.

The chronic pulmonary eosinophilia in *C. neoformans*-infected CCR2\(^{-/-}\) mice was confirmed by both histological and flow-cytometric analysis. At 6 wk postinfection, lungs from both CCR2\(^{+/+}\) and CCR2\(^{-/-}\) mice were fixed, sectioned, and stained with hematoxylin and eosin. The lungs of infected CCR2\(^{+/+}\) mice exhibited only localized areas of cellular infiltration, whereas mice lacking CCR2 had extensive areas of infiltration (Fig. 5, A and B). High power examination of CCR2\(^{+/+}\) mouse lungs revealed that the localized infiltrate was mainly mononuclear (Fig. 5C), whereas affected areas in CCR2\(^{-/-}\) mouse lungs contained numerous cryptococci, eosinophils, and crystalline deposits (Fig. 5D). These crystals are derived from eosinophil degranulation following interaction with *C. neoformans* during a T2-type response (12). The complex granular structure of the eosinophil lends itself to identification by flow cytometry by causing increased side light scatter.

**Lack of CCR2 results in delayed CD8\(^+\) T cell recruitment**

Analysis of pulmonary lymphocytes by flow cytometry revealed changes in lymphocyte subsets even though total lymphocyte numbers were not different between infected CCR2\(^{+/+}\) and CCR2\(^{-/-}\) mice. Pulmonary CD8\(^+\) T cells were reduced in CCR2\(^{-/-}\) mice at weeks 1 and 2 compared with CCR2\(^{+/+}\) mice (reductions of 48% and 32%, respectively) (Fig. 6). By week 3, there was no difference and, by week 6, CCR2\(^{-/-}\) mice had twice the number of CD8\(^+\) T cells in the lung compared with CCR2\(^{+/+}\) mice. There were no significant differences in pulmonary CD4\(^+\) T cells and B220\(^+\) B
cells throughout the course of infection, although there was a trend toward higher numbers of B cells in the lungs of CCR2−/− mice by week 6. Thus, CCR2 is involved in early recruitment of CD8 T cells into the lung during pulmonary C. neoformans infection.

C. neoformans infection induces a T2-type immune response in the absence of CCR2

The possibility that C. neoformans-infected CCR2-deficient mice produce a polarized T2-type immune response was further investigated by examining cytokine production, anticyclococcal DTH, and serum IgE. Leukocytes were isolated from infected lungs and cultured for 24 h without additional stimulus. Culture supernatants were then harvested and analyzed for IL-4, IL-5, and IFN-γ production by ELISA. Analysis of cytokine production profiles revealed a distinct difference between CCR2+/+ and CCR2−/− mice (Fig. 7). Leukocytes from CCR2+/+ mice produced significant amounts of the type 1 cytokine IFN-γ beginning at week 1 and continuing through week 3, while production of the type 2 cytokines IL-4 and IL-5 remained minimal. Inversely, leukocytes from CCR2−/− mice showed marked production of IL-4 and IL-5 beginning at week 2 with no production of IFN-γ. A similar T1/T2 polarization pattern was seen when infected mice were tested for the development of DTH (T1) and production of IgE (T2) (Fig. 8). Footpad injection of C. neoformans filtrate Ag produced a characteristic DTH response in CCR2+/+ mice, but not in CCR2−/− mice. Although the lack of a DTH response is consistent with a T2-type response in CCR2−/− mice, it is possible that reduced DTH in these mice could be attributed to a reduction in CCR2-dependent macrophage recruitment. IgE levels remained uninflected levels in CCR2+/+ mice, but were dramatically increased in the serum of infected CCR2−/− mice at 3 and 6 wk. These results demonstrate that expression of CCR2 is required for the development of a T1 response to C. neoformans infection and lack of CCR2 results in a T2-type response.

Discussion

The present study has demonstrated that CCR2 expression is required for the development of a T1- over a T2-type immune response in the lung following cryptococcosis infection. Intratracheal inoculation of C. neoformans into mice lacking CCR2 produced a prolonged pulmonary infection and significant dissemination to the spleen and brain that were not seen in CCR2-expressing mice. In addition, CCR2 deficiency resulted in significantly reduced pulmonary recruitment of macrophages and CD8+ T cells during the first 2–3 wk of infection. In contrast to the T1-type response generated in CCR2-expressing mice, CCR2-deficient mice produced a strong T2 immune response to pulmonary C. neoformans infection. The immune response in CCR2−/− mice was characterized by chronic pulmonary eosinophilia, crystal deposition in the lungs, pulmonary leukocyte production of IL-4 and IL-5 but not IFN-γ, and increased serum IgE. These results demonstrate that expression of CCR2 is required for the development of a T1-type response to C. neoformans infection and lack of CCR2 results in a switch to a T2-type response.

These results demonstrate a novel role for CCR2 in mediating T1 vs T2 switching to an infectious agent. Chemokines might promote immune response polarization by directing selective migration of either Th1 or Th2 cells to the site of infection (13). This idea is based on the distinct expression of certain chemokine receptors, such as CXCR3/CXCR5 for Th1 cells and CCR3/CCR4 for Th2 cells (13). However, Th1 and Th2 cells both express CCR2 (13), and CCR2−/− mice are defective in their ability to develop T1- or T2-mediated lung granulomas elicited by embolization of Ag-coated beads (8, 14). Chemokines may also promote immune...
response polarization by modulating Th1 vs Th2 lymphocyte differentiation (15, 16). Neutralization of CCR2 ligand MCP-1 decreased IL-4 and increased IFN-γ production by CD4+ T cells cocultured with lung fibroblasts or splenic macrophages (15). Similarly, OVA-specific TCR transgenic T cells stimulated through the TCR and treated with MCP-1 had enhanced IL-4 production (16). However, splenocytes from CCR2 or MCP-1 knockout mice are also defective in their ability to produce IFN-γ (8, 17). Our earlier work with C. neoformans infection showed that MCP-1 is required for the efferent phase of the T1-type response against this pathogen (3). Our present results are consistent with this earlier observation and show further that in the absence of CCR2, the primary MCP-1 receptor, there is a switch in Th polarization.

CCR2+/- mice have defects in the ability to control a C. neoformans infection. Could this inability to control the infection lead to a Th2 response? Infectious burden or Ag levels can be a determinant in T1 vs T2 responses (18). However, there is no difference in organism burden through week 2 in the lungs, spleen, or brain between CCR2+/- and CCR2−/- mice. Therefore, the mechanism of T1 to T2 switching in CCR2−/- mice is not due to a difference in early infectious burden.

Defective macrophage and CD8+ T cell recruitment/activation in CCR2-deficient mice is a possible mechanism that could drive a T1 to T2 switch in the immune response to C. neoformans. We have previously shown that depletion of CD8+ T cells during C. neoformans infection results in the production of predominantly T2-type cytokines by CD4+ T cells (4). Therefore, IFN-γ production by CD8+ T cells is most likely important for the development of Th1-type CD4+ T cell immunity to C. neoformans. It remains to be determined whether the CD8+ T cell defect observed in the lungs of CCR2−/- mice is also observed in the lung-associated lymph nodes, the likely site of T1/T2 differentiation during pulmonary C. neoformans infection (19). Activated macrophages are an important source of cytokines, such as IL-12, that influence a developing T1 response in the lungs and lymph nodes. IL-12 has been shown to induce IFN-γ-dependent increases in both mononuclear cell infiltration and MCP-1 production, resulting in a protective response to pulmonary C. neoformans infection (20, 21). Because monocytes and macrophages express CCR2 (14) and lack of CCR2 expression prevents monocyte/macrophage recruitment into the lungs following C. neoformans infection, it is possible that there are recruitment/activation defects of these cells and other APC in the lymph nodes. Thus, our data suggest that there may also be changes in the lymph nodes of CCR2−/- that account for T2-type response to C. neoformans in these mice.

The lack of IFN-γ production in the lungs of C. neoformans-infected CCR2-deficient mice is likely to contribute to the switch to a T2 response. Previous studies using cryptococcal infection models have demonstrated the importance of IFN-γ production during the development of protective T1-type immune response (19, 22–25). Induction of IFN-γ in CCR2+/- mice (week 1) precedes the induction of IL-4 in CCR2−/- mice (week 2), suggesting that production of IFN-γ during a C. neoformans infection down-regulates induction of IL-4 and prevents a T2 response. However, preliminary studies in our lab have demonstrated that there are factors in addition to IFN-γ involved in driving the T1-type response. Neutralization of IFN-γ at the onset of a pulmonary C. neoformans infection in CCR2+/- mice did not duplicate the phenotype exhibited by infected CCR2−/- mice (data not shown). At 2 wk postinfection, anti-IFN-γ-treated mice had reduced cryptococcal clearance, increased eosinophil recruitment, and a trend toward greater leukocyte IL-5 production similar to that seen in infected CCR2-deficient mice (data not shown). However, early IFN-γ neutralization did not decrease macrophage or CD8+ T cell recruitment, nor did it increase IL-4 production (data not shown). Therefore, the switch to a T2 response in CCR2-deficient mice cannot be attributed solely to a defect in IFN-γ production.

Lack of cryptococcal clearance in CCR2-deficient mice is not due to decreased production of the CCR2 ligand, MCP-1. Infected CCR2-deficient mice had 3-fold greater levels of MCP-1 in BAL fluid at 1-wk postinfection and increased MCP-1 production by cultured leukocytes compared with control mice. In addition, analysis of whole lung homogenates for MCP-1 production in uninfected mice revealed that basal MCP-1 production was 4-fold greater in CCR2−/- mice (≤250 and 1218 pg/llung for CCR2+/- and CCR2−/-, respectively, unpublished observations). Increases in lung mRNA have been observed for the CCR2 agonists MCP-1, MCP-3, and MCP-5 during schistosomal Ag-elicited pulmonary granuloma formation (14). The finding that MCP-1 production is greater in the absence of CCR2 suggests that CCR2 is involved in a negative feedback loop responsible for attenuating both basal and stimulated MCP-1 production.

The high levels of MCP-1 in C. neoformans-infected CCR2-deficient mice could be a factor in the switch to a T2-type immune response. MCP-1 transgenic mice are more susceptible to infection by intracellular pathogens, and it was suggested that high levels of MCP-1 stimulated a Th2-dominant response (4). Furthermore, MCP-1 plays an important role in the generation of a T2-type response in models such as schistosome egg Ag-induced lung granuloma (14) and experimental autoimmune encephalomyelitis (26–28). Therefore, in the absence of CCR2, it is possible that the high levels of MCP-1 seen during C. neoformans infection might stimulate a T2 response via a second receptor, such as CCR9 (29) or an as yet unidentified receptor for MCP-1 (30).

Fungal infections can elicit protective T1- or nonprotective T2-type immune responses. Our data indicate that the absence of CCR2 results in a strong T2-type response to the fungus C. neoformans that fails to clear the infection. In addition, CCR2 is required for macrophage and CD8+ T cell recruitment to the site of infection. Thus, CCR2 expression, along with induction of the cytokines TNF-α, IL-12, and IFN-γ, is critical for the development of T1- over T2-type antifungal immunity.

Acknowledgments

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References


